IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (previously presented) A process for preparation and purification of recombinant human interferon (hu-IFN) alpha 2b which comprises:

I. cultivating recombinant *Pichia pastoris* containing a hu-IFN alpha 2b gene,

II. culturing said recombinant *Pichia pastoris* in complex/defined salt culture medium to produce hu-IFN alpha 2b protein, and

III. purifying recombinant hu-IFN alpha 2b protein from said culture medium.

2. (currently amended) A process as claimed in claim 1 wherein said human IFN alpha 2b gene comprises [[()]SEQ ID NO: 3[[)]].

3. (previously presented) A process as claimed in Claim 1 wherein said recombinant *Pichia pastoris* containing a hu-IFN alpha 2b gene is cultivated by first isolating and purifying mRNA from human leucocytes, preparing a first strand of DNA from said purified mRNA to obtain said modified hu-IFN alpha 2b gene, amplifying said gene and cloning said amplified modified hu-IFN alpha 2b gene into an expression vector, amplifying and isolating said hu-IFN alpha 2b gene from said modified interferon alpha 2b clone, cloning said hu-IFN alpha 2b gene into an expression vector and transforming said expression vector into said *Pichia pastoris*.

4. (currently amended) A process as claimed in claim 1 wherein said cloning is carried out by RT-PCR ~~methods~~ employing primer pairs having the sequences selected from the group consisting of SEQ ID NOS: 4 & 5; 6 & 7; 8 & 9; 10 & 11; 12 & 13 and 12 & 14.

5. (currently amended) A process as claimed in Claim 3 wherein said *Pichia pastoris* is selected from the group consisting of *Pichia pastoris* KM 71, *Picha pastoris* KM 71H, *Pichia pastoris* GS115, and *Pichia pastoris* X33 ~~preferably *Pichia pastoris* KM71~~.

6. (original) A process as claimed in Claim 3 wherein said vector is pPICZαA.

7. (previously presented) A process as claimed in Claim 6 wherein said hu-IFN alpha 2b gene is cloned in pPICZαA vector down stream to AOX promoter and alpha mat signal sequence.

8. (previously presented) A process as claimed in Claim 3 wherein a desired construct containing a hu-IFN alpha 2b gene (expression cassette) is integrated at the AOX region of *Pichia pastroris*.

9. (currently amended) A process as claimed in Claim 8 wherein said expression cassette is integrated at the 5' AOX region of a *Pichia pastoris* selected from the group consisting of *Pichia pastoris* KM 71, *Picha pastoris* KM 71H, *Pichia pastoris* GS115, and *Pichia pastoris* X33 ~~preferably *Pichia pastoris* KM71~~.

10. (previously presented) A process as claimed in Claim 9 wherein said *Pichia pastoris* has His auxotrophic phenotype.

11. (currently amended) A process as claimed in Claim 1 wherein said culture medium is selected from the group consisting of ~~complex media like~~ BGY, BY, BMY, BGYP, YPD, and defined salt medium ~~preferably defined salt medium and BMY~~.

12. (previously presented) A process as claimed in Claim 11 wherein said culture medium comprises one or more nitrogen sources selected from the group consisting of ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, bean-cakes, potato extracts, protein hydrolysates, yeast extract, urea and ammonium hydroxide.

13. (currently amended) A process as claimed in Claim 1 wherein said culture medium comprises a carbon source selected from the group consisting of ~~such as~~ glycerol, glucose, fructose, and methanol ~~and the like, preferably glycerol~~.

14. (previously presented) A process as claimed in Claim 1 wherein the biomass build up is in a range of 35 to 100 g/L, preferably 35-50 g/L for complex medium and 50 to 80 g/L, preferably 50-60 g/L for defined salt medium based on dry cell weight.

15. (previously presented) A process as claimed in claim 14 wherein said culture medium has:

(a) pH in the range of 3.0 to 6.0, preferably 6.0 to 6.5 for complex medium and 3.5 to 4.5 for defined salt medium preferably 5.8 to 6.2,

(b) temperature in the range of 25 to 35°C preferably 28 to 32°C, and

(c) dissolved oxygen: 20-80% of saturation, preferably 40-50% of saturation and said culturing is carried out for a duration of 48 to 110 hours, preferably 48 to 72 hours for complex medium and 90-110 hours for defined salt medium.

16. (currently amended) A process as claimed in Claim 11 wherein the expression of recombinant IFN alpha 2b protein is induced after reaching appropriate biomass buildup using a suitable alcohol ~~such as methanol, ethanol and the like preferably methanol~~ at a concentration of 0.1 to 3.0% v/v~~, preferably 1-1.5% v/v~~.

17. (currently amended) A process as claimed in Claim 16, wherein the expression of full length recombinant IFN alpha 2b protein is regulated by addition of a nitrogen source selected from the group consisting of yeast nitrogen base, yeast nitrogen base without amino acid, yeast hydrolysate, yeast extract, peptone, casamino acid, meat extract, and beef extract ~~and like, preferably yeast extract and peptone~~ along with or without propylene glycol.

18. (currently amended) A process as claimed in Claim 1 wherein said recombinant hu-IFN alpha 2b protein is purified to homogeneity by

(a) separating cells from the cell culture to obtain the supernatant which contains recombinant hu-IFN alpha 2b protein;

(b) subjecting said supernatant to cation exchange chromatography by

(i) binding said recombinant hu-IFN alpha 2b protein on a column packed with CM SEPHAROSE FF, SP SEPHAROSE FF or SEPRAPREP S,

(ii) washing said column with a buffer selected from the group consisting of citrate, phosphate, acetate buffer, and [[or]] CIEXI buffer, at a pH 5.0-5.5 to remove unwanted proteins, and

(iii) eluting said recombinant hu-IFN alpha 2b protein with CIEXII buffer with pH 4.8-5.4;

(c) subjecting the eluent obtained in step (b)(iii) to anion exchange chromatography followed by elution with AIEX II buffer;

(d) subjecting the eluent from step (c) to ultrafiltration with membrane of pore size 10,000 Dalton molecular cut off to obtain a concentrated retentate containing recombinant hu-IFN alpha 2b protein;

(e) subjecting said concentrated retentate to gel filtration chromatography using ammonium acetate buffer containing Tween-80 and EDTA, pH 5.2-5.5, to obtain homogenous species of recombinant hu-IFN alpha 2b protein; and

(f) purifying said recombinant hu-IFN alpha 2b protein obtained in step (e) by repeating steps (a) to (e) in any sequence or order.

19. (previously presented) A pharmaceutical composition comprising purified interferon alpha 2b prepared and purified according to claim 1, and a pharmaceutically acceptable carrier either in liquid form or in lyophilized form.

20. (previously presented) A pharmaceutically composition as claimed in Claim 19 wherein said pharmaceutically acceptable carrier comprises phosphate buffer, glycine, HSA, PEG, ammonium acetate, NaCl, Tween-80, EDTA, Benzyl alcohol and the like in any combination and with desired concentration / amount.

21. (previously presented) A method of treatment using purified interferon alpha 2b prepared and purified according to claim 1 for treatment of viral diseases like chronic active Hepatitis B, Chronic active Hepatitis non A-non B, Chronic active Hepatitis delta, Chronic active Hepatitis C; cancer diseases like Chronic myelogenous leukemia, Non-

Hodgkin's lymphoma, AIDS related Kaposi's Sarcoma, Renal cell carcinoma, Malignant melanoma, Hairy cell leukemia, Bladder carcinoma, Superficial and noduloulcerative basal cell carcinoma, Condylomata acuminata, Laryngeal papillomatosis, and like.